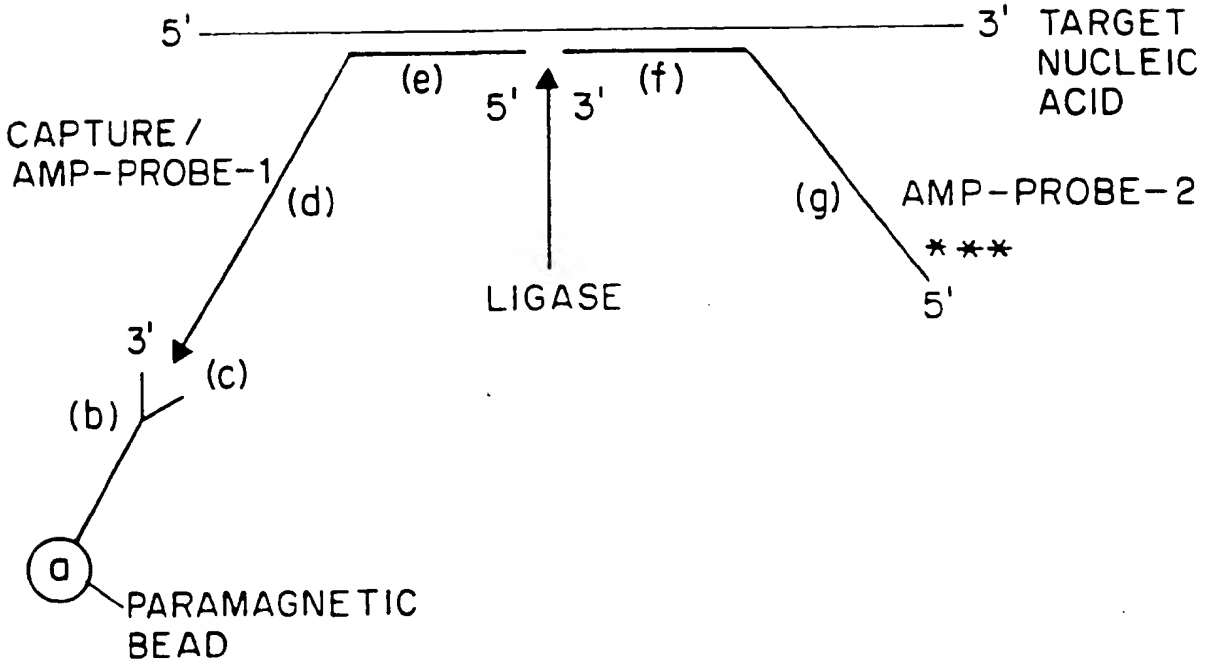
5'
3'
TARGET NUCLEIC ACID
(e)
(f)
5'
3'
CAPTURE / AMP-PROBE-1
(d)
(g)
AMP-PROBE-2
***
LIGASE
5'
3'
(c)
(b)
a
PARAMAGNETIC BEAD

FIG. 1

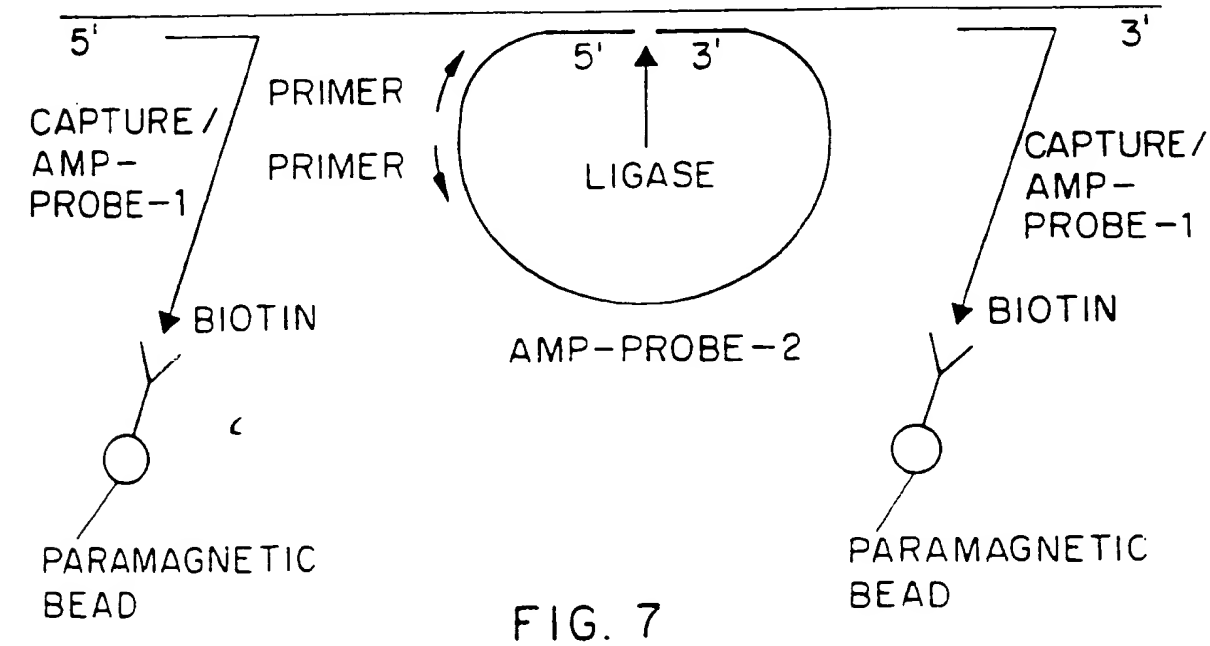
5'
3'
5'
3'
CAPTURE / AMP-PROBE-1
PRIMER
PRIMER
LIGASE
CAPTURE / AMP-PROBE-1
BIOTIN
AMP-PROBE-2
BIOTIN
PARAMAGNETIC BEAD
PARAMAGNETIC BEAD

FIG. 7

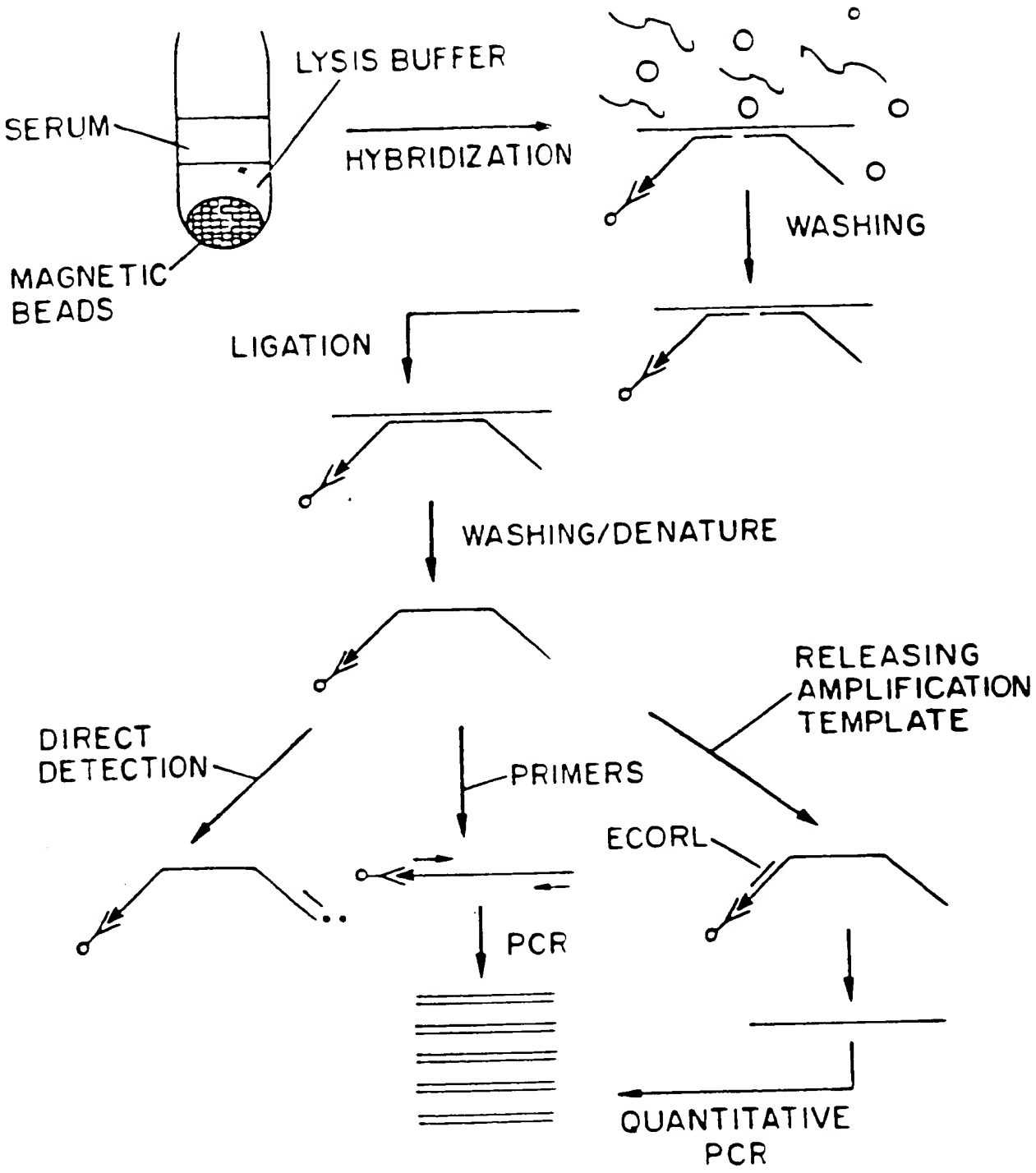
LYSIS BUFFER
SERUM
HYBRIDIZATION
MAGNETIC BEADS
WASHING
LIGATION
WASHING/DENATURE
RELEASING AMPLIFICATION TEMPLATE
DIRECT DETECTION
PRIMERS
ECORL
PCR
QUANTITATIVE PCR

FIG. 2

Document Number 2,165,545

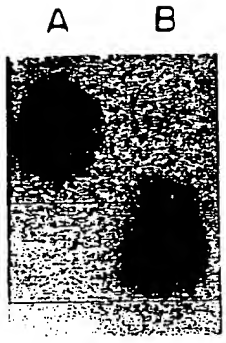

FIG.3

T0910T T928260

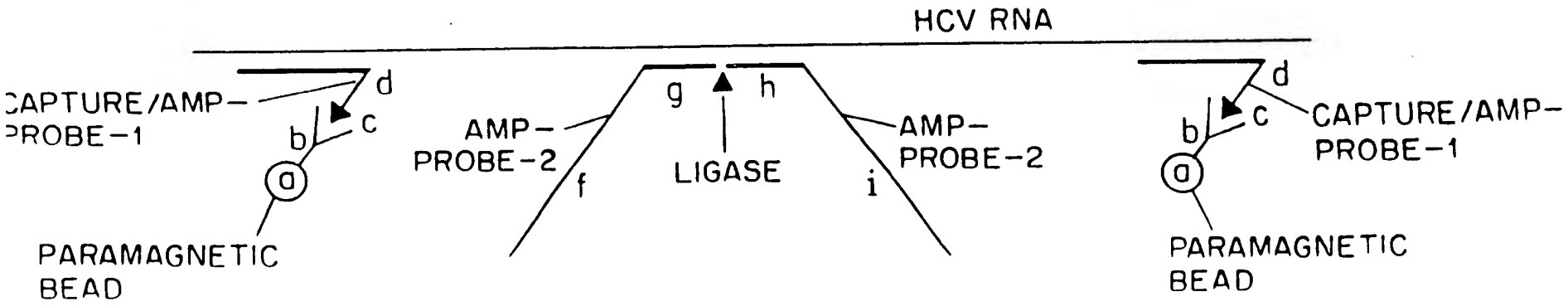

FIG. 4

(4 of 24)

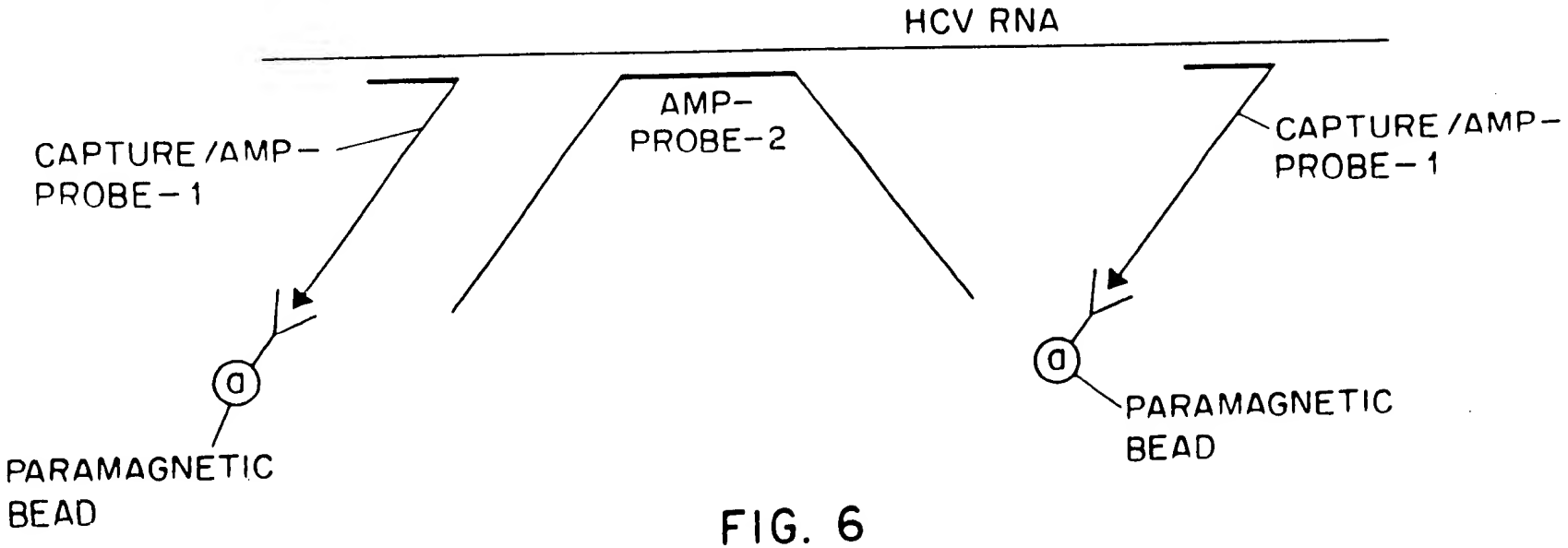

FIG. 6

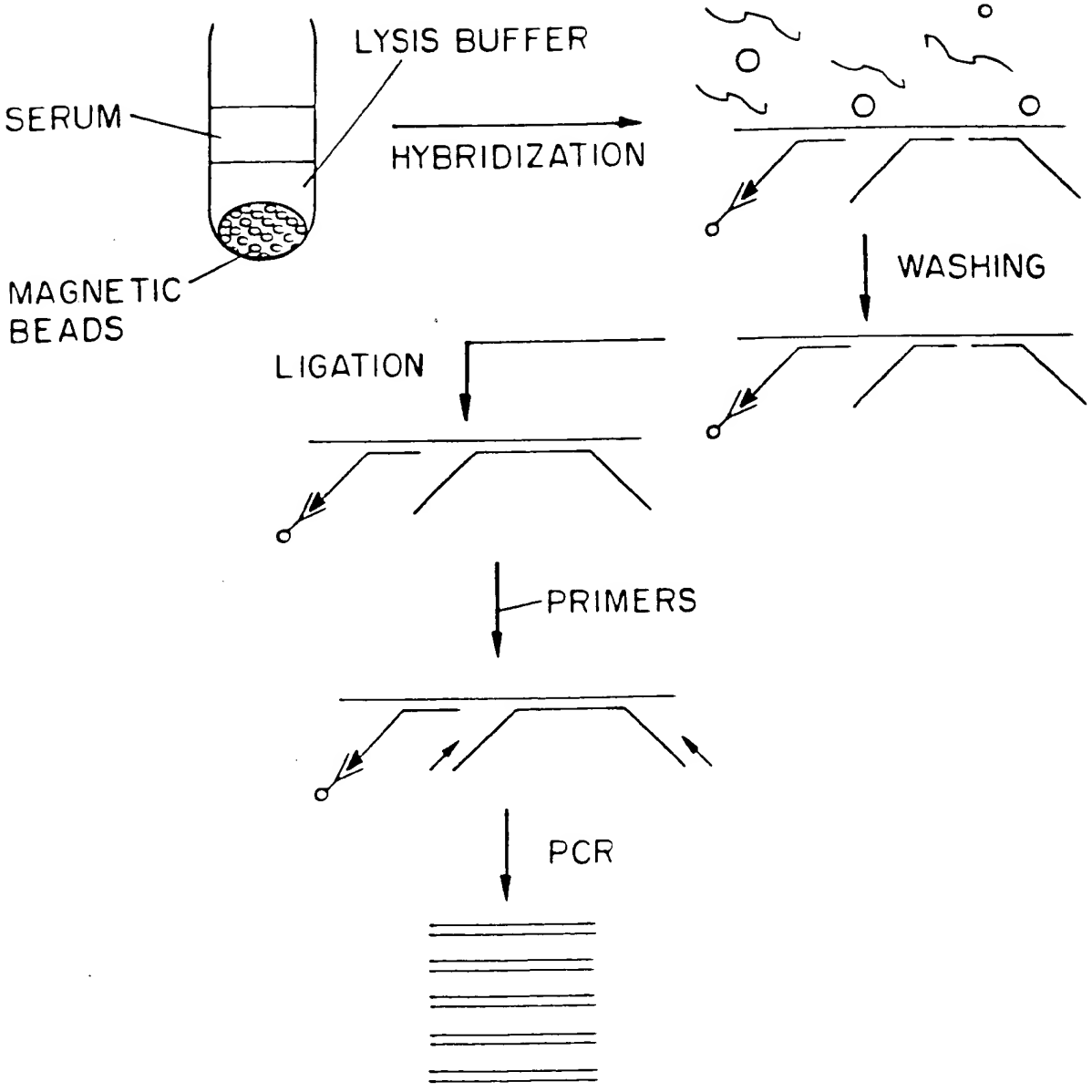
LYSIS BUFFER
SERUM
HYBRIDIZATION
MAGNETIC BEADS
WASHING
LIGATION
PRIMERS
PCR

FIG. 5

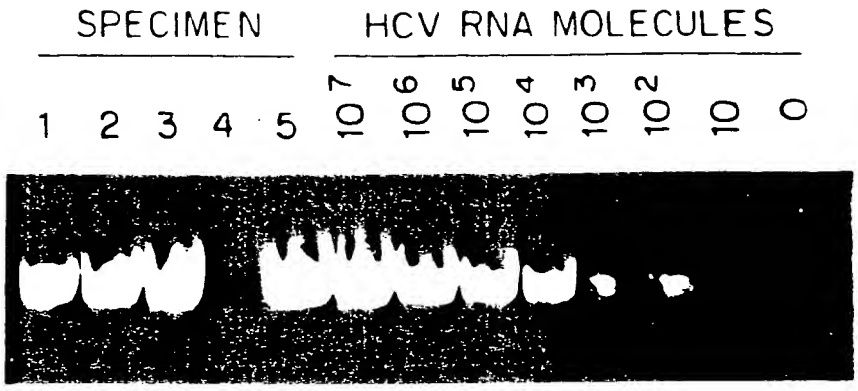
SPECIMEN
HCV RNA MOLECULES
1 2 3 4 5
10^7 10^6 10^5 10^4 10^3 10^2 10 0

FIG.8

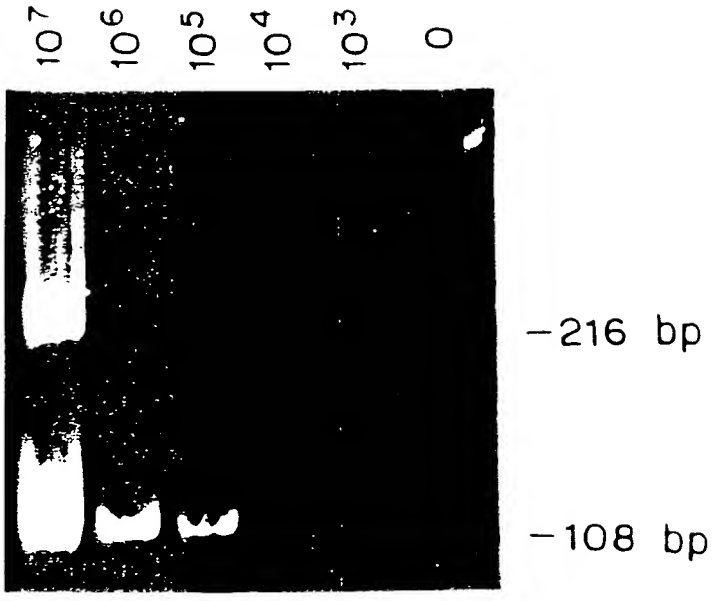
10^7 10^6 10^5 10^4 10^3 0
−216 bp
−108 bp

FIG.9

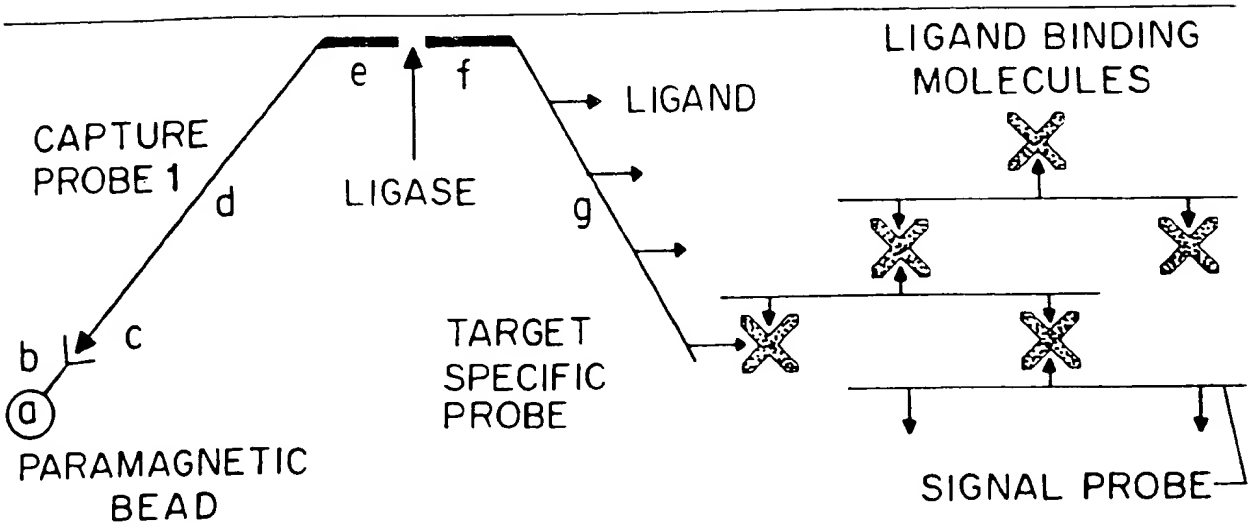

FIG. 10

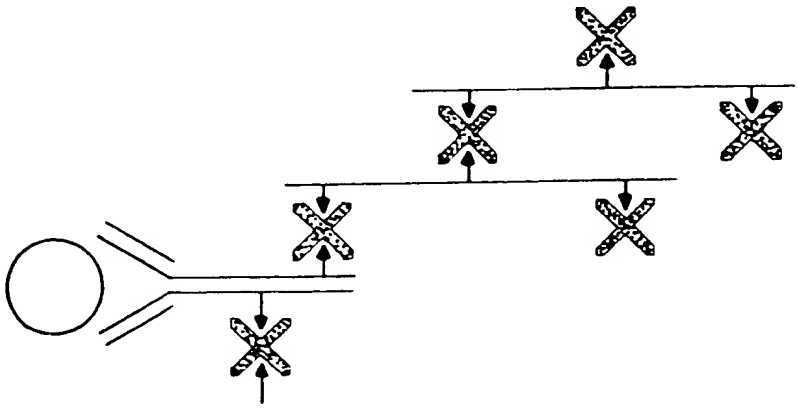

FIG. 11

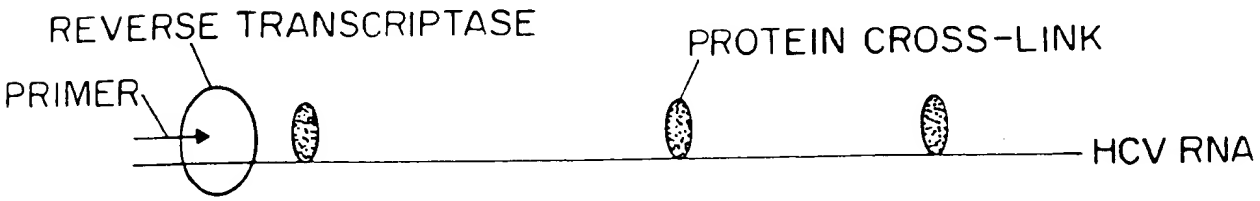

FIG. 12A

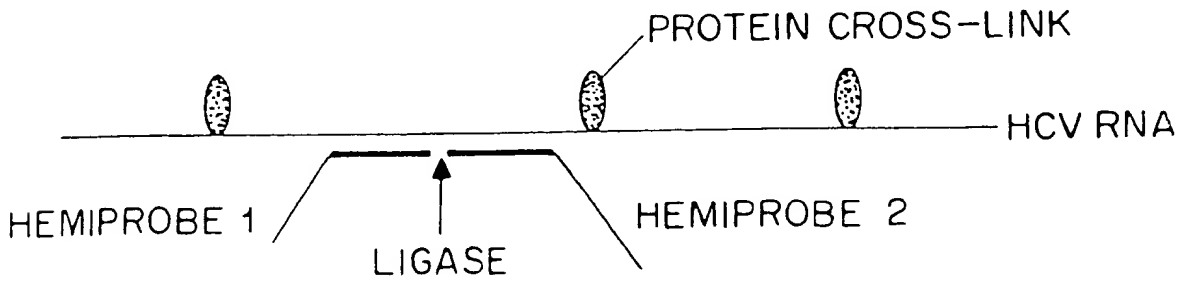

FIG. 12B

24545
(8 of 24)

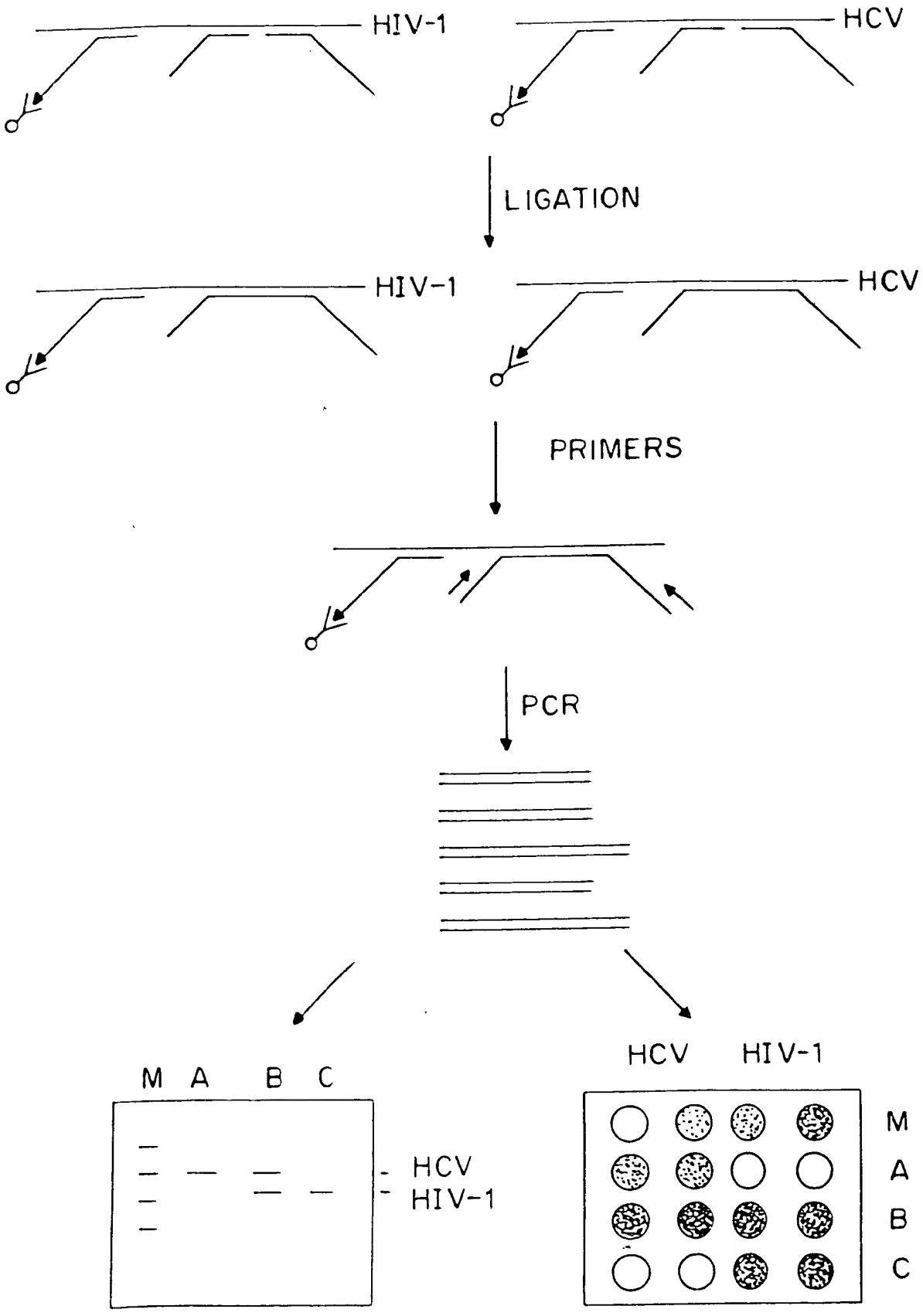

FIG 13

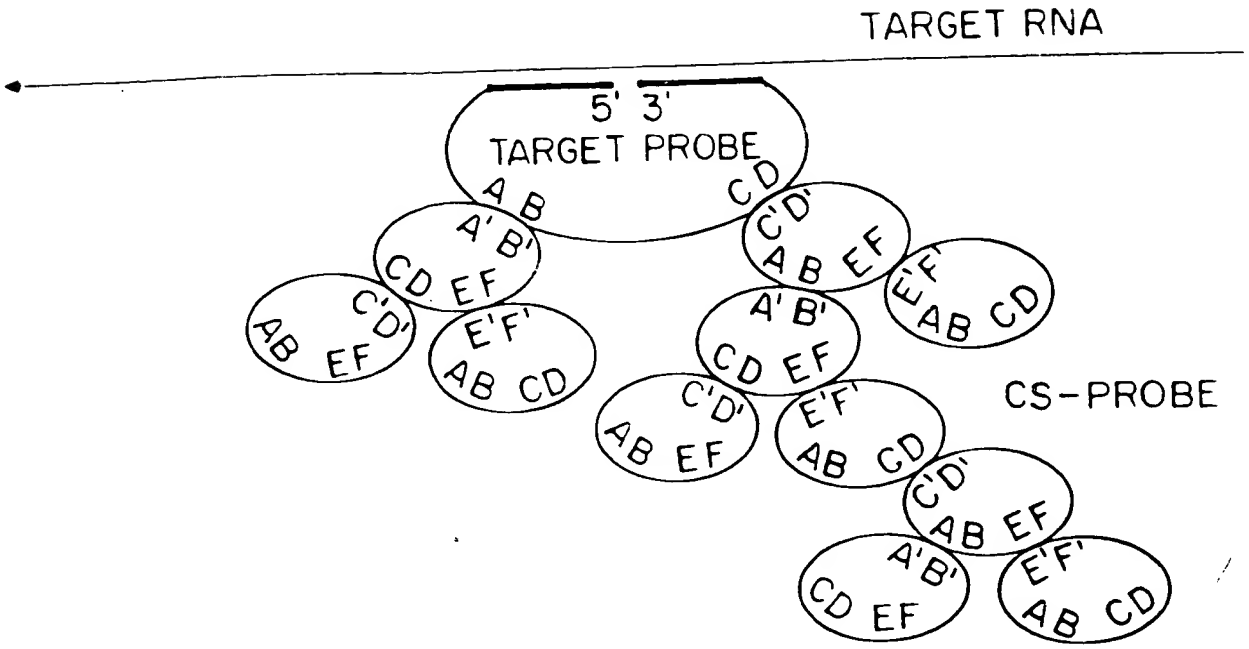
TARGET RNA
5' 3'
TARGET PROBE
A B
C D
A' B'
CD EF
C'D'
AB EF
E'F'
AB CD
C'D'
AB EF
A'B'
CD EF
E'F'
AB CD
CS-PROBE
C'D'
AB EF
E'F'
AB CD
A'B'
CD EF
E'F'
AB CD

FIG. 14

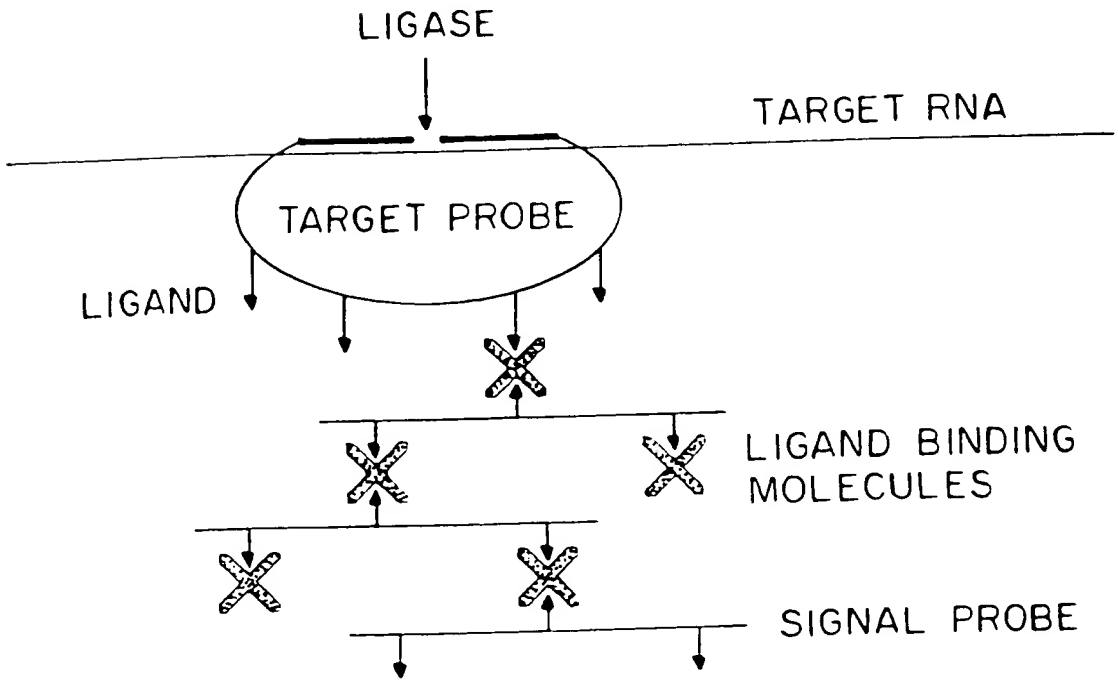
LIGASE
TARGET RNA
TARGET PROBE
LIGAND
LIGAND BINDING
MOLECULES
SIGNAL PROBE

FIG. 15

DOSIOT'T9282660

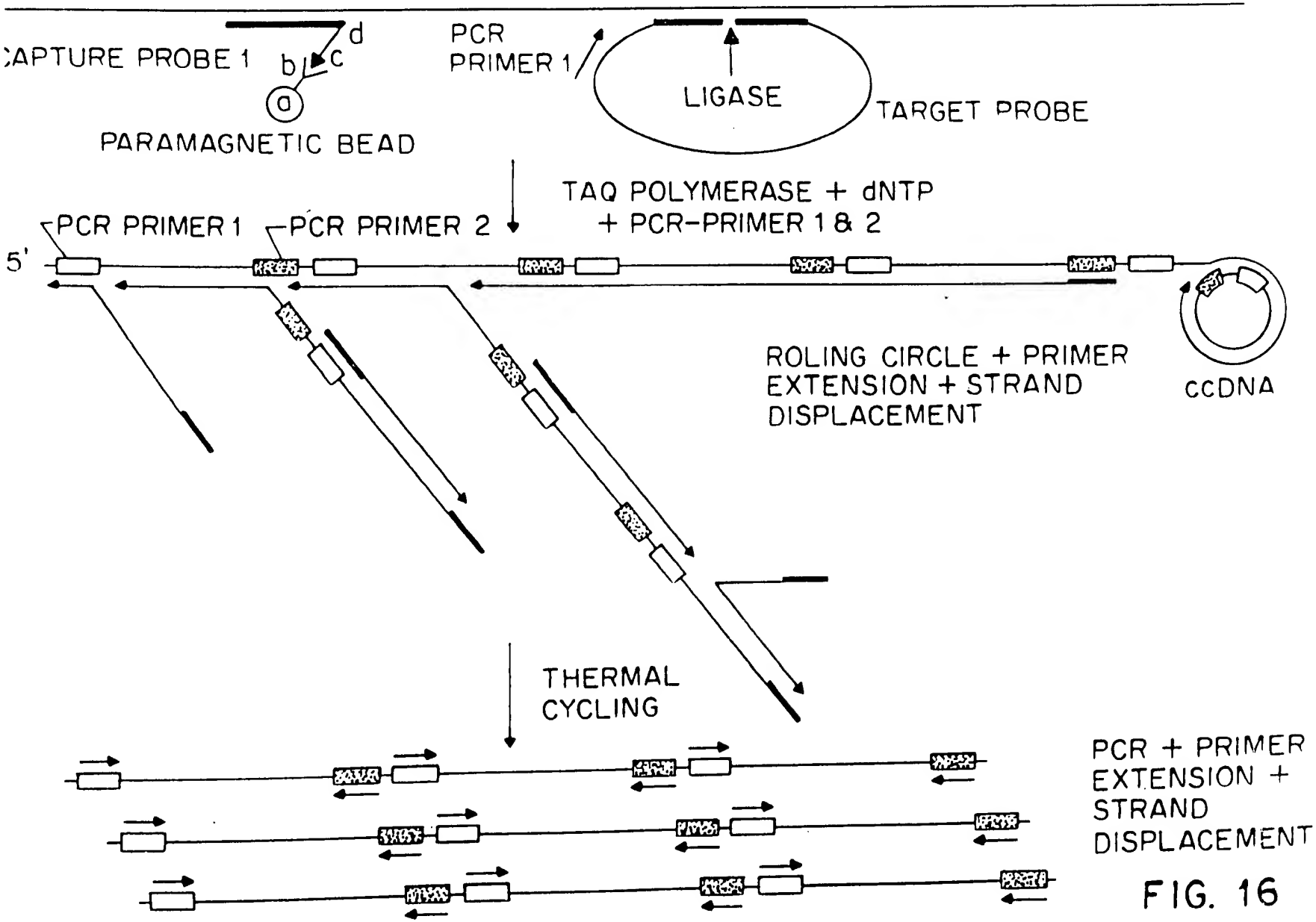

FIG. 16

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(10 of 24)

Without Ligation

With Ligation

FIG. 18

T0910T T9287660

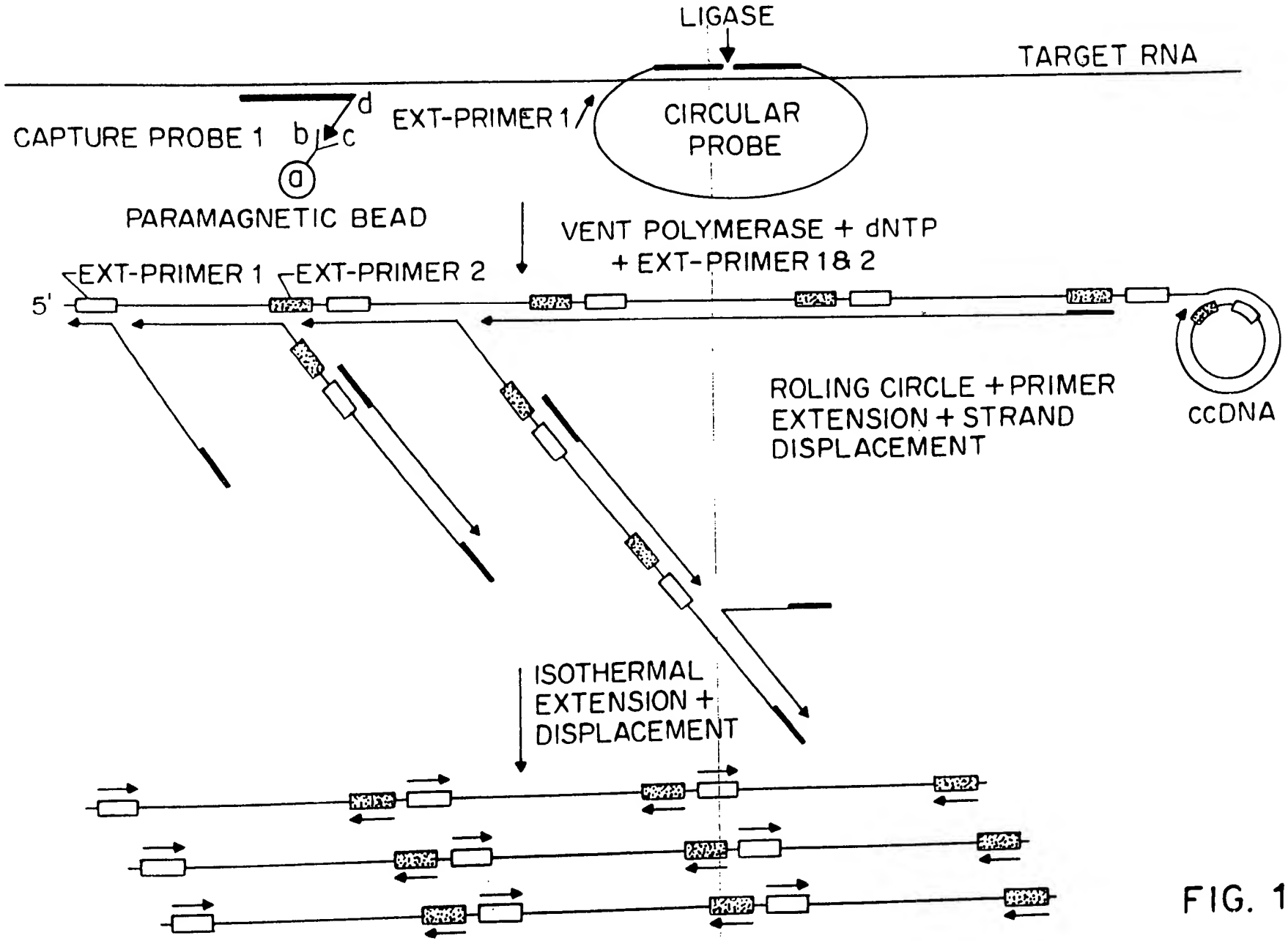


FIG. 19

12 of 24
36545

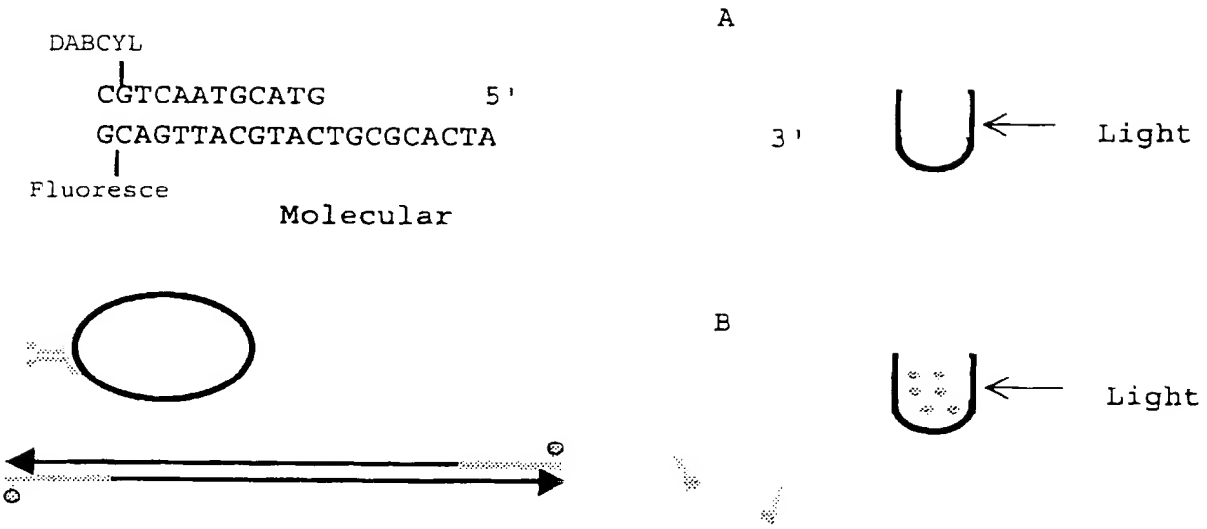
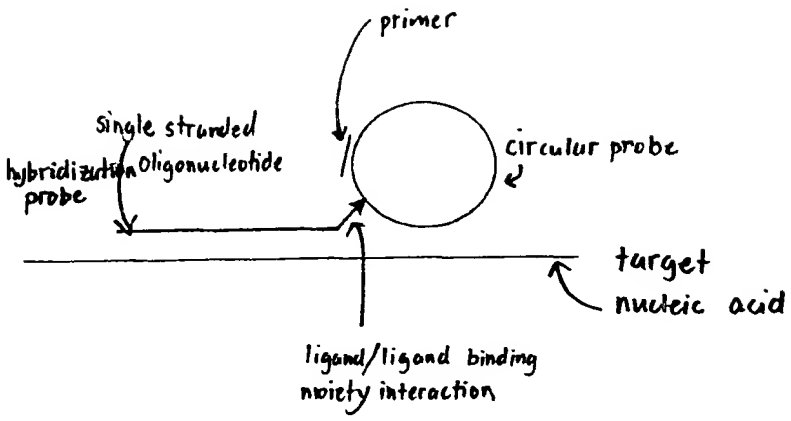

DABCYL
CGTCAATGCATG 5'
GCAGTTACGTACTGCGCACTA 3'
Fluoresce
Molecular
A
Light
B
Light

Fig. 20


primer
single stranded
hybridization probe
oligonucleotide
circular probe
target
nucleic acid
ligand/ligand binding
moiety interaction

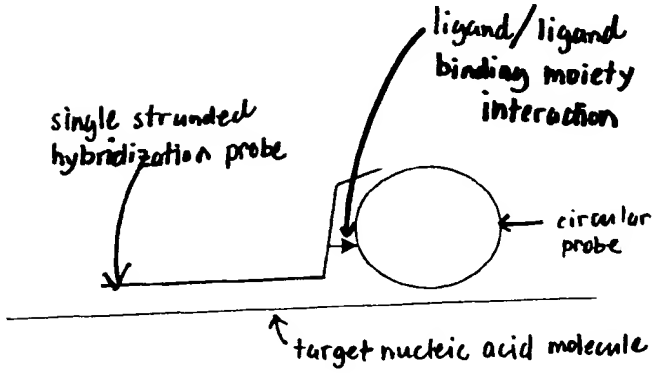
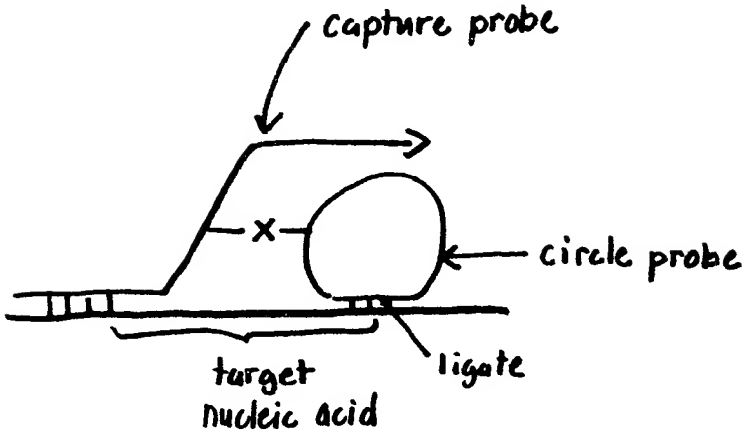
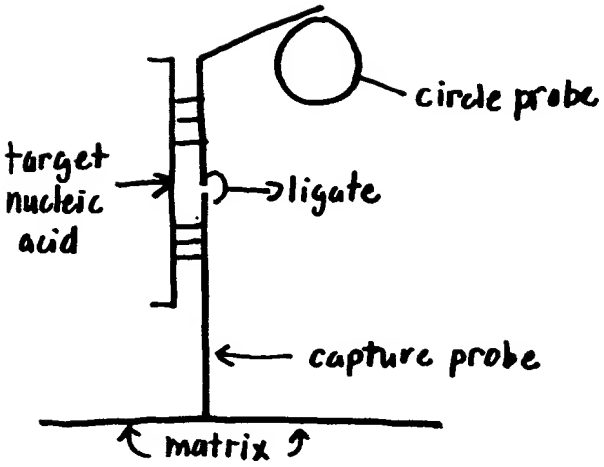

ligand/ligand
binding moiety
interaction
single stranded
hybridization probe
circular
probe
target nucleic acid molecule

Figure 21 A-B



21 C



21D

Figure 21 C-D

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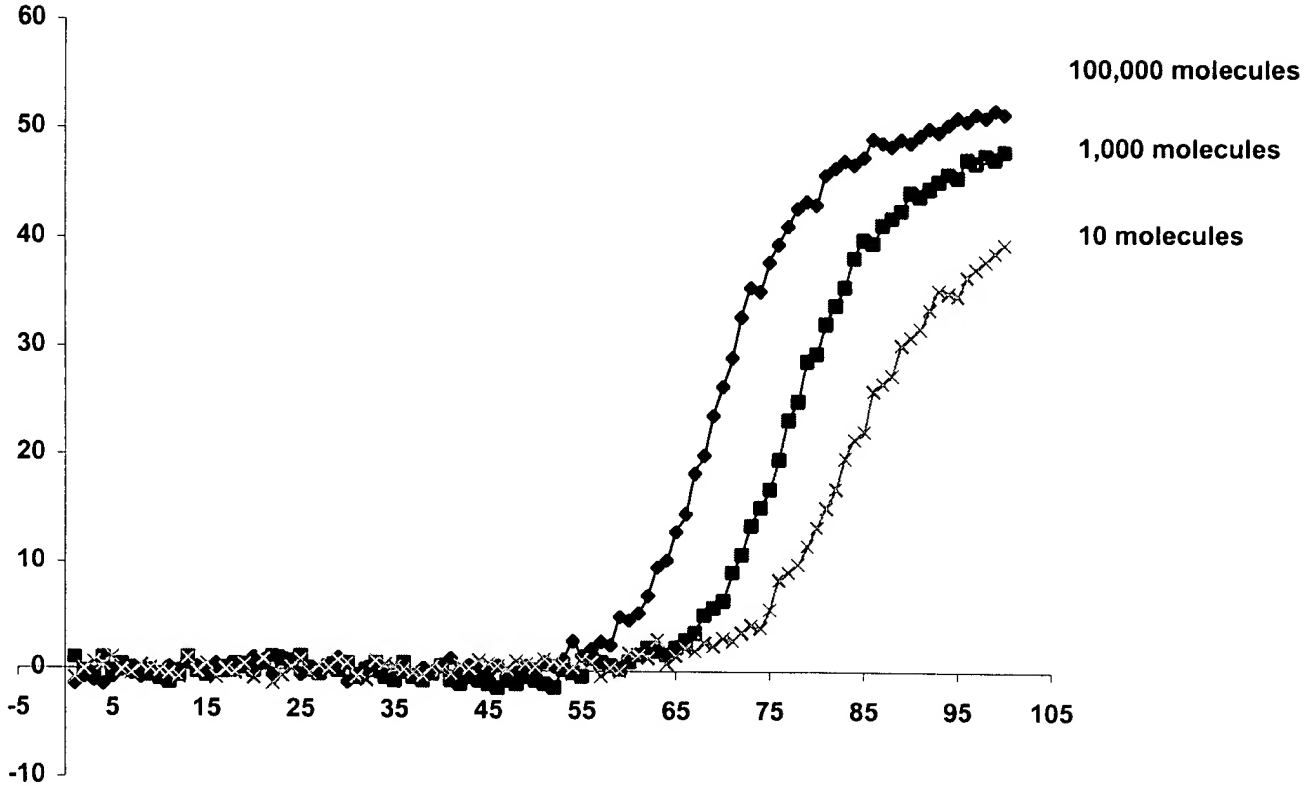

Figure 22

Inhibition of biotin-avidin on RAM (Bst/P29)

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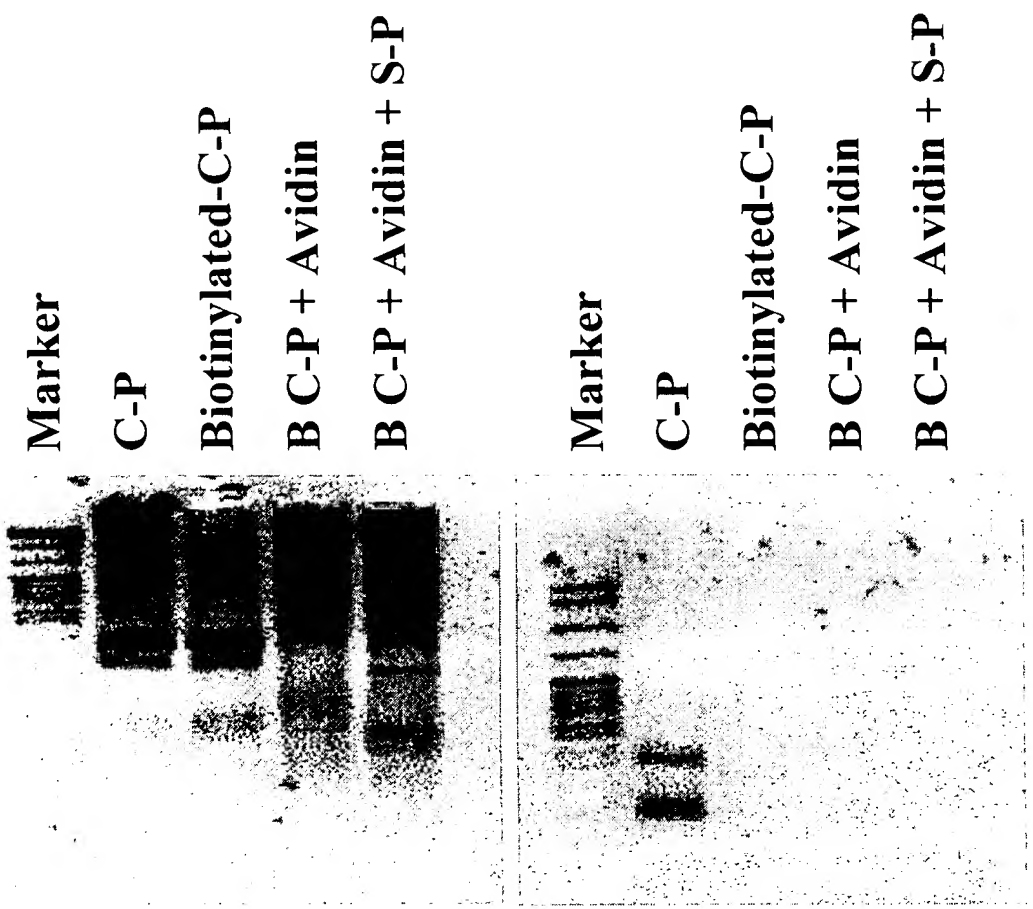
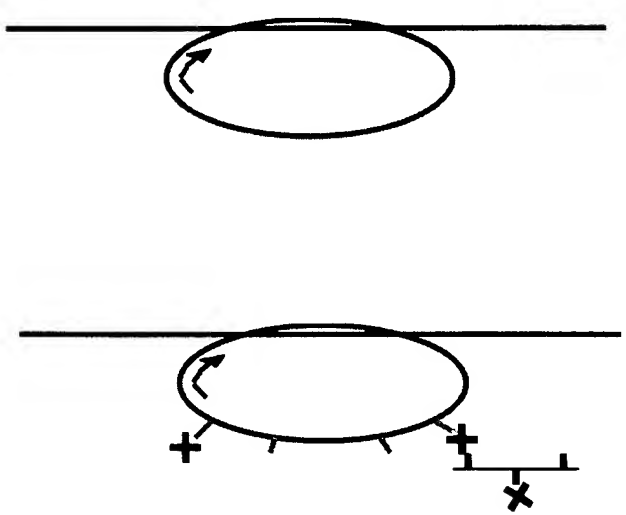
Figure 23



Bst polymerase **φ 29 polymerase**



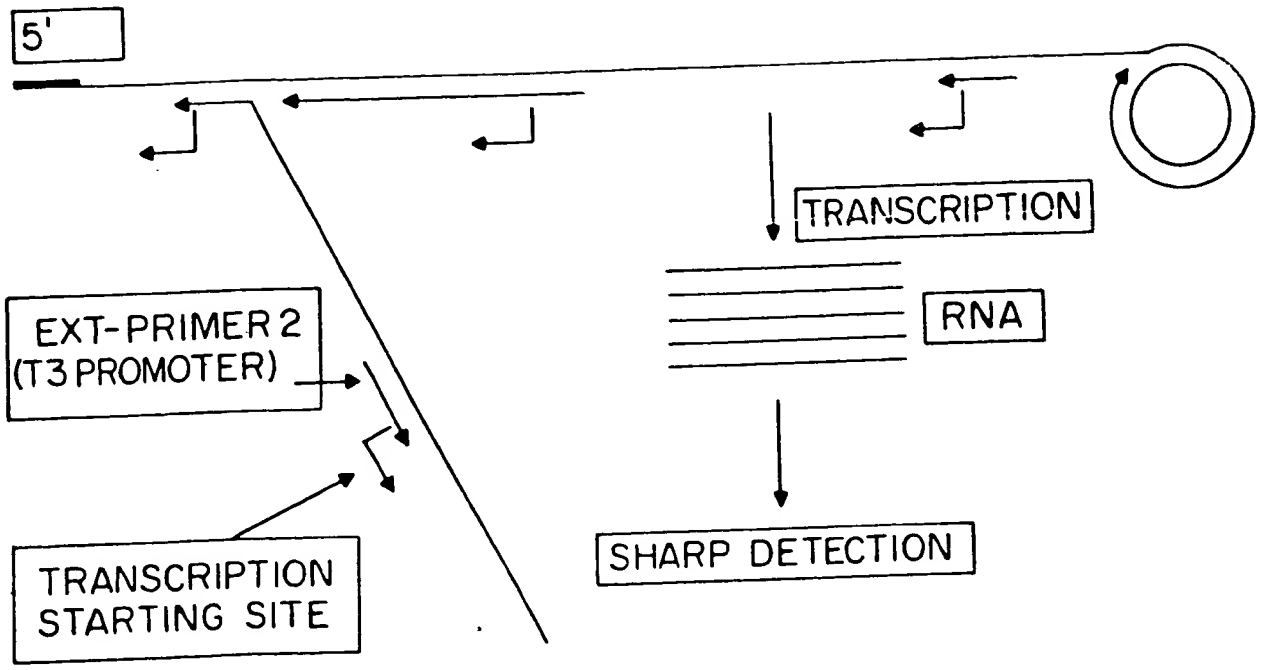

5'
TRANSCRIPTION
RNA
EXT-PRIMER 2
(T3 PROMOTER)
TRANSCRIPTION
STARTING SITE
SHARP DETECTION

FIG. 17

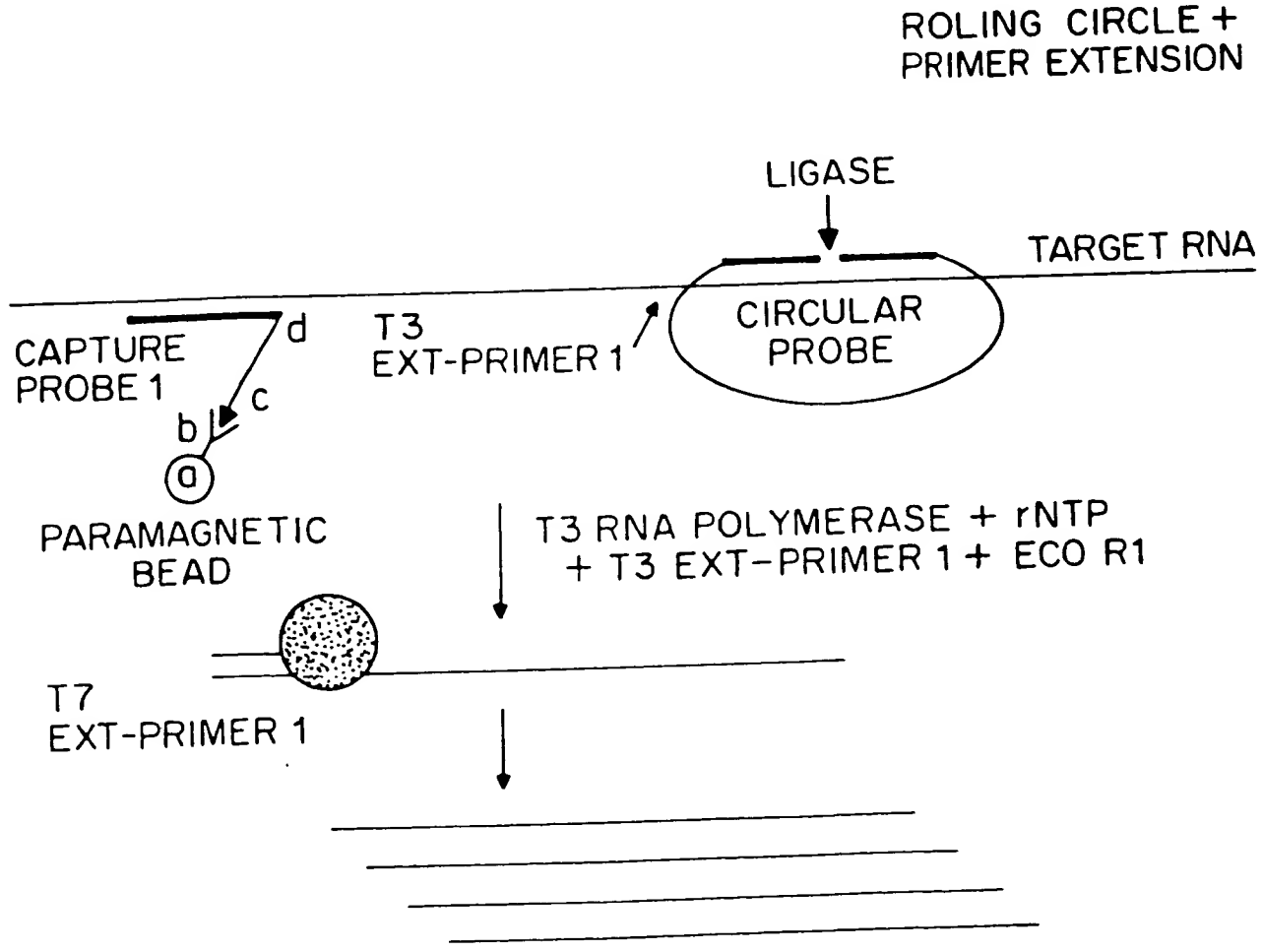

ROLING CIRCLE +
PRIMER EXTENSION
LIGASE
TARGET RNA
CIRCULAR
PROBE
T3
EXT-PRIMER 1
CAPTURE
PROBE 1
d
c
b
a
PARAMAGNETIC
BEAD
T3 RNA POLYMERASE + rNTP
+ T3 EXT-PRIMER 1 + ECO R1
T7
EXT-PRIMER 1

FIG. 24

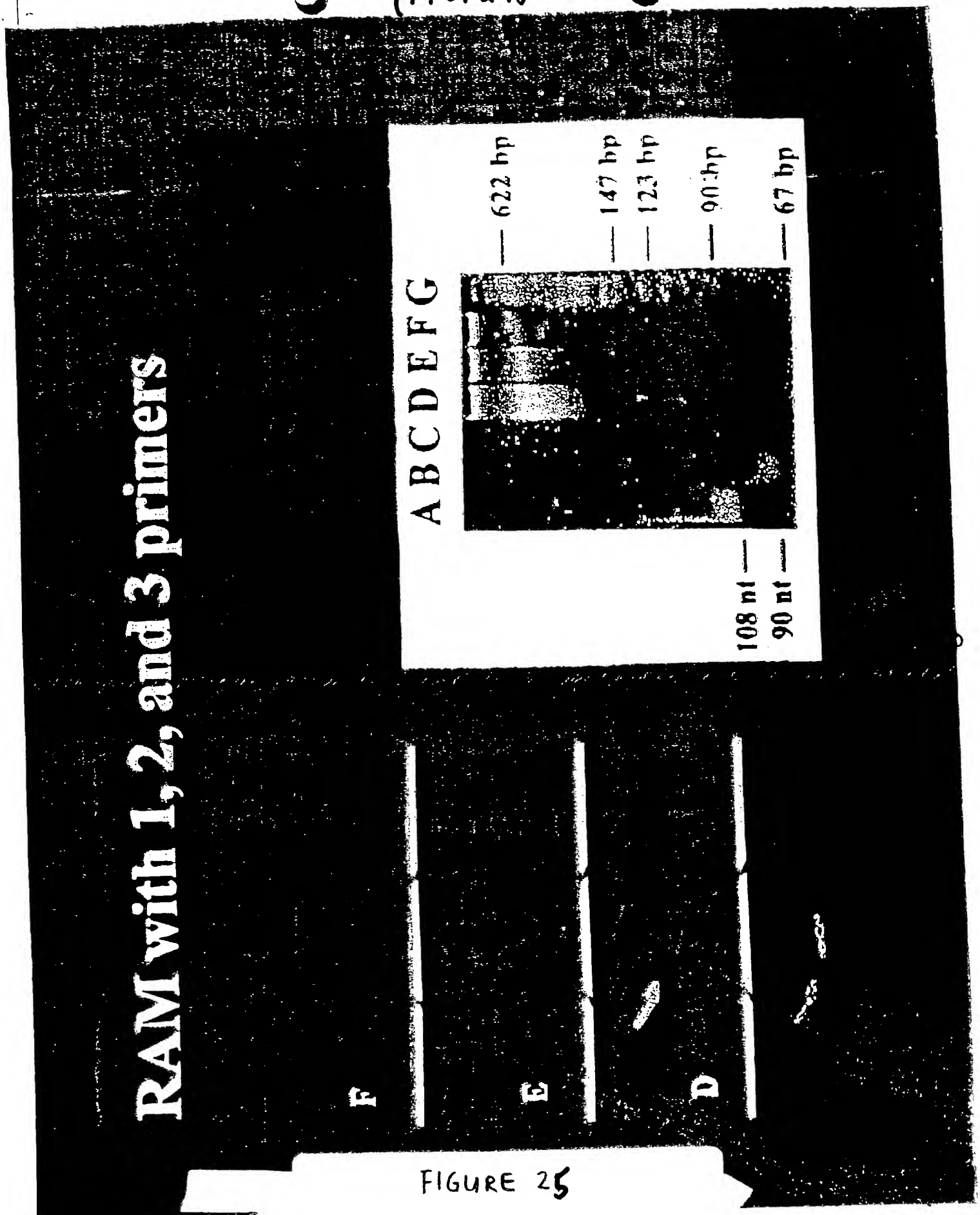

RAM with 1, 2, and 3 primers
F
E
D
ABCDEFG
622 bp
147 bp
123 bp
90 bp
67 bp
108 nt
90 nt

FIGURE 25

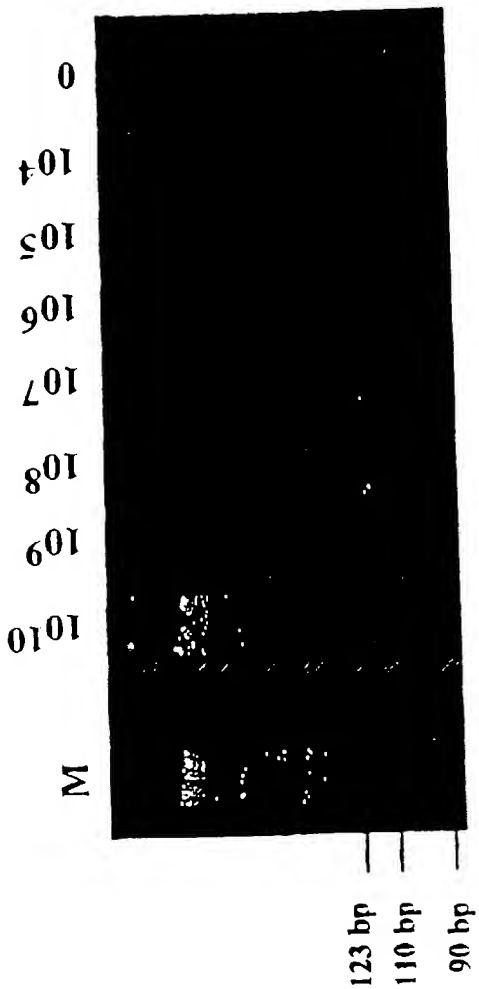


FIG. 26

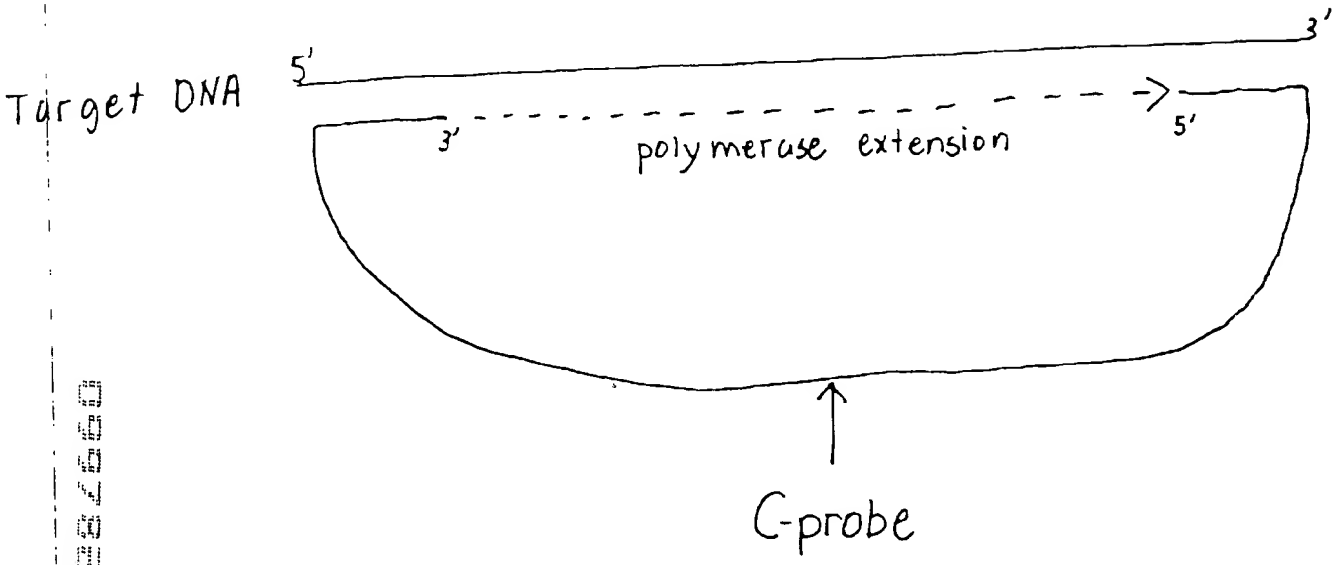
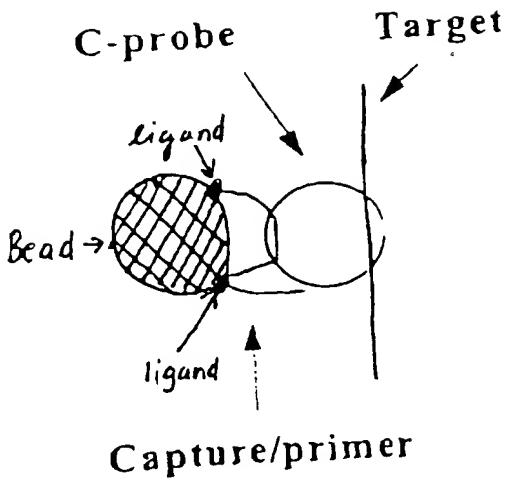


FIG 27


C-probe
Target
ligand
Bead
ligand
Capture/primer

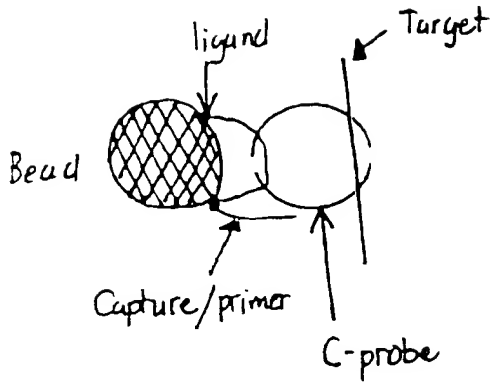
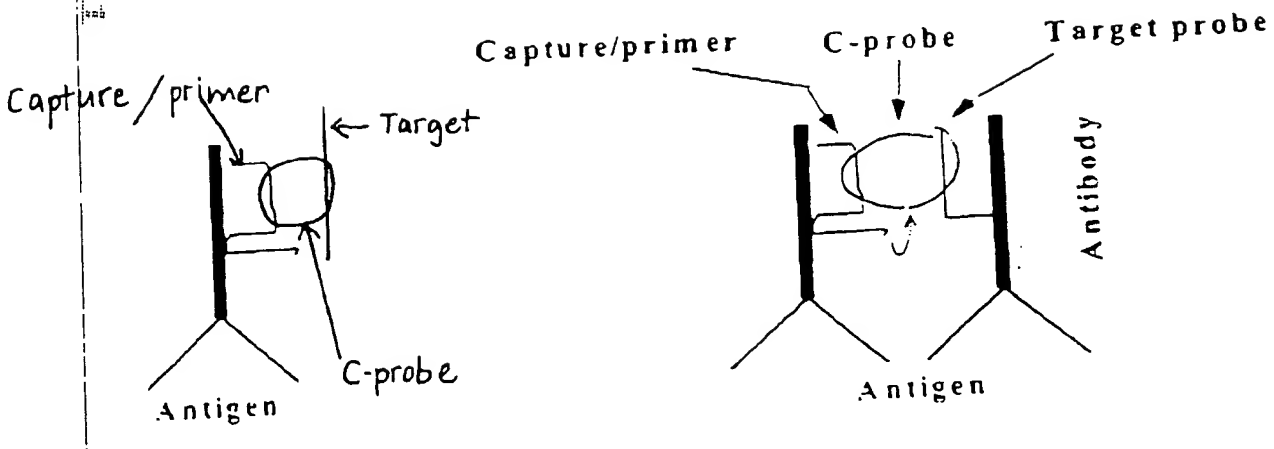

ligand
Target
Bead
Capture/primer
C-probe

Figure 28

FIGURE 29


Capture/primer
Target
C-probe
Antigen
Capture/primer
C-probe
Target probe
Antibody
Antigen

POSTOT 1988660

NOV 22 2000 13:07

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(24 of 24)

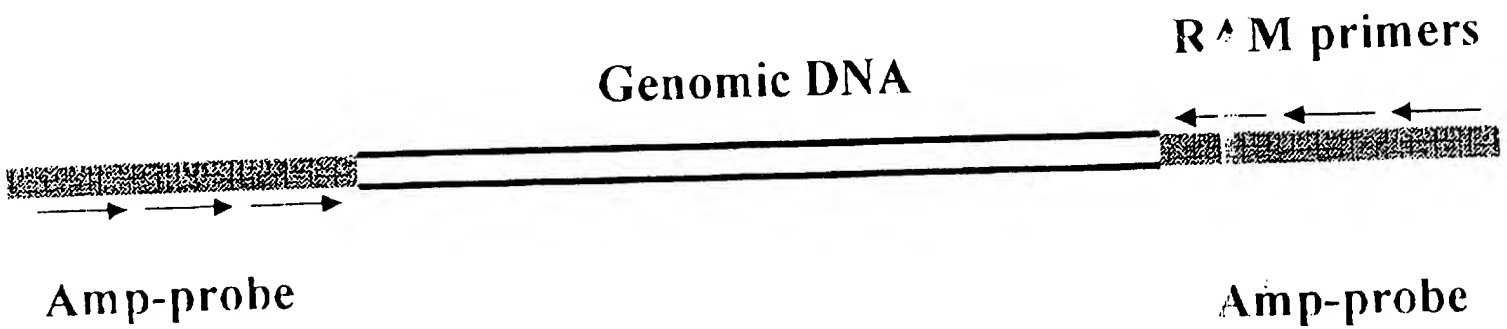

Figure 30

TOTAL P.02
PAGE.02